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Increasing the iron content of hay grown on soils producing nutritional anemia in Massachusetts livestock

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INCREASING THE IRON CONTENT OF HAY GROWN ON SOILS PRODUCING NUTRITIONAL ANEMIA IN MASSACHUSETTS LIVESTOCK

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INCREASING THE IRON CONTENT OF HAY CROPS ON SOILS PRODUCING NUTRITIONAL ANEMIA IN MASSACHUSETTS LIVESTOCK

by

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INTRODUCTION

HISTORY AND OCCURRENCE OF NUTRITIONAL ANEMIA IN LIVESTOCK.

During the winter of 1933-34 a peculiar disease of cattle which had given considerable trouble for a good many years to Massachusetts farmers living around Buzzards Bay and vicinity, was first brought to the attention of the Agricultural Experiment Station. After a careful investigation by Freeman[1] and Archibald[2], the ailment was diagnosed as a "nutritional anemia".

Although this particular sickness with cows has just been apprehended, it has been an age-old problem in the area surrounding Buzzards Bay. Farmers of the region call it "neck ail". The towns of Marion, Wareham, Sandwich and Barnstable are places where the disease has been reported and in which investigation has been undertaken.

"Nutritional anemia" of cattle, although for the most part limited in Massachusetts to the south-eastern region, has long been a problem with cattle grazing on certain types of soil in the state of Florida. In that state there are certain large areas where it is almost, if not totally, impossible to raise cows profitably.

[1] [illegible]. L. Freeman, field agent for the Eastern Grain Co., Bridgewater, Massachusetts.

[2] J. G. Archibald, research professor in Animal Husbandry, at the Massachusetts Agricultural Experiment Station.

The malady is not confined to certain areas of Massachusetts and Florida. Other parts of the world have previously reported the same disease or a very similar one. In New Zealand both sheep and cows have been extensively affected when grazed on ranges with soils of a certain pumice formation. Kings' Island, Tasmania; Kenya Colony, British East Africa; and sections along the Scottish border are other regions from which reports show the presence of nutritional anemia.

SYMPTOMS OF ANEMIC CATTLE.

Archibald of the Massachusetts State Experiment Station gives the following description of the anemic cattle. The sick animal loses its appetite, refusing food given it, especially grain. It takes on a listless look and becomes thin. The head of the affected cow looks as if only the hide covered the skull. Flesh and weight is lost. In some cases the hair of the animal is shed in large patches. The hemoglobin content of the blood of the affected animals is reduced. Some cases were found in which it was as low as 61% of normal.

Nearly all the cattle in the regions surrounding Buzzards Bay have been affected to varying degrees by this nutritional sickness. Becker, Neal and Shealy (9) of Florida and Aston (3,4) of New Zealand describe an affected animal as one which has lost its appeite, often refusing to eat what has been offered it, but readily eats weeds, dirt, rags and other refuse matter. The sick animal looks run down and the mucous membrane, liver, kidney and blood are pale in color. The hemoglobin content as well as the volume of the blood is greatly diminished. The weight of a young animal does not increase and animals in good condition lose considerable flesh and look sickly. Sexual maturity is delayed and reproduction interfered with. If curative methods are not practiced, the affected animal dies in surroundings of apparent plenty.

Figure 1 is a photograph of a young anemic heifer at Marion, Massachusetts. The animal has lost considerable flesh and hair.

THE CAUSE OF NUTRITIONAL ANEMIA IN MASSACHUSETTS.

It seems that people living in regions where the cattle become affected by nutritional anemia learned, a long time ago, how to cope with the malady if it was not too far advanced. They observed that cattle grazing on certain pastures having a characteristic soil type or formation would be affected with the disease, while animals grazing on other pastures adjoining or relatively near, would not be affected. By moving affected cattle during the grazing season, from a "sick" area into an area which did not produce the sickness, they could be cured. Strangely enough, the cattle men of all the regions mentioned above where cows become anemic, practiced

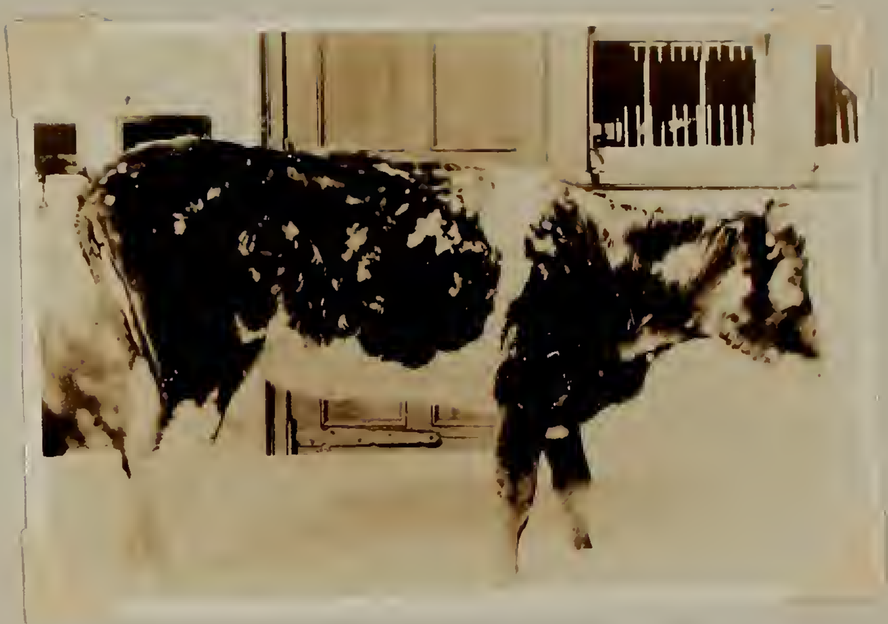

Figure 1

Yearling heifer at Marion, Massachusetts. Picture taken in March 1934. She was in even worse condition than this before the "drench" began to show results.

Figure 2

Same heifer as in Figure 1, at Marion, Massachusetts in May 1934. She had received the "drench" of ferric ammonium citrate from February on.

this cure for the sickness long before scientific investigation had been undertaken.

From analyses of the soil from the "sick area" around Buzzards Bay and the grass grown upon it, Brooke of the Wirthmore Research Laboratory, found that both the soil and grass were exceedingly low in iron content. The examination of the blood of affected animals fed on this grass showed a low iron content. If affected animals were treated with a "drench"[1] of ferric ammonium citrate, recovery to a healthy condition was soon noted. Figure 2 is a photograph of the same animal shown in Figure 1, after it had received such a "drench" for three months. The recovery is very striking.

These observations and the similarity of the symptoms to those in Florida pointed to an iron deficiency in the feed as the probable cause of the trouble. This in turn was seemingly due to the lack of available iron in the sandy soils of the region, thus making the iron content of the forage very low. Cattle eating this forage with a low iron content do not obtain enough iron to supply their needs for blood and body building.

[1] Drench is the term applied to the internal administration of medicinal liquids to sick livestock.

OTHER NAMES FOR THE NUTRITIONAL ANEMIA IN CATTLE.

In New Zealand the disease of cattle and sheep caused by seemingly iron deficiency is called "bush sickness" or "skinnies". Sheep in the Cheviot Hills in the southern part of Scotland which become anemic are said to have the "pining", while the term "nakuruitis" is used to designate a similar disease of sheep in the Nakuru district of Kenya Colony. On King Island, Tasmania "coasty disease" is claimed to be the same nutritional trouble among livestock. In Florida the disease is named locally either as "salt sick", "hay sick", "cow sick", "hill sick", "marsh sick", "prairie sick", "scrub sick" or "the sick".

REVIEW OF LITERATURE.

Salt sick has been studied in Florida for over forty years by the Agricultural Experiment Station. In 1930, corrective measures were discovered and applied by Becker, Neal and Shealy (9). These same authors attributed the sickness to a deficiency of iron in feeds and associated it with certain types of greyish sandy soil. They found that forage grown on these sick areas contained less iron than those grown on other healthy areas. They further state that if cattle are grazed on the deficient areas for approximately six to ten months and then moved and allowed to graze on surrounding healthy areas, the disease can be controlled or avoided to a large degree. Cattle owners, therefore, move their cattle from range to range to enable them to utilize their pastures and keep their cattle in a healthy condition at the same time. On "salt sick" soil areas, the authors recommended the placement of "licks" to control the disease. These are made by placing in a box a mixture of 100 pounds of common salt, 25 pounds of red oxide of iron and 1 pound of copper sulphate.

Figures 3, 4 and 5 are photographs of animals, submitted with the permission of Professor R. B. Becker and the Florida Agricultural Experiment Station, which were taken in Florida and used in this paper in order to show the similarity of condition of sick animals in Florida to those in Massachusetts.

In Figure 3 the animal shows the typical emaciation and gauntness observed in advanced cases of "salt sick", or nutritional anemia. Since this picture was taken in 1930, the cow has recovered, has remained healthy, and has reproduced annually (1932-1937), while having access to iron-copper supplement on the same iron deficient range.

Figure 4 is of a calf showing an advanced stage of "salt sick", or nutritional anemia. Note emaciation, lack of condition as indicated by the hair, appearance of the eye and that the animal shows evidence of diarrhoea.

Figure 3

This animal shows the typical emaciation and gauntness observed in advanced cases of "salt sick", or nutritional anemia. Since this picture was taken in 1930, she recovered, has remained healthy, and has reproduced annually (1932-1937) while having access to iron-copper supplement on the same deficient range.

Figure 4

A calf showing an advanced stage of "salt sick", or nutritional anemia. Note emaciation, lack of condition as indicated by the hair, appearance of the eye, and that the animal shows evidence of diarrhea. (Cf. Fig. 5.)

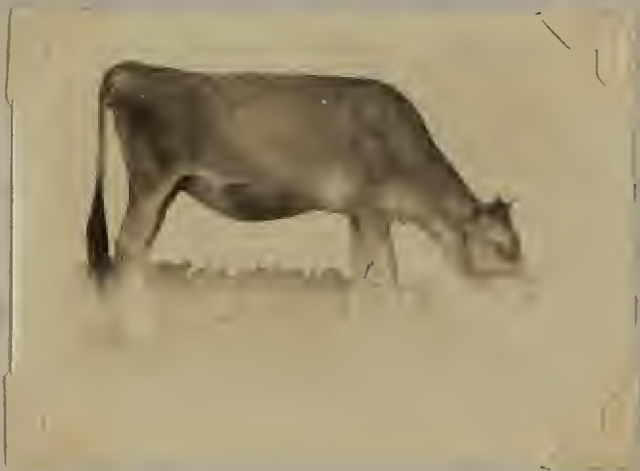

Figure 5

The calf shown in Figure 4, recovered when given access to the iron-copper supplement. In advanced stages, a more soluble form of iron was used. In this instance, bonemeal was added to the salt sick lick, for the soil area also lacked phosphorus.

Figure 5 shows the same calf as that in Figure 4 after it had recovered when given access to the iron-copper supplement. In advanced stages, a more soluble form of iron was used. In this instance, bonemeal was added to the "salt sick lick", for the soil area also lacked phosphorus.

Neal and Becker (15) noted that the affected ranges consisted of white and greyish sand, cumulose muck lands and shallow residual soils overlying marl. The soils of the healthy ranges were red and yellow sands, clays, shallow sands underlaid with clay, and soils subject to overflow from clay lands. They also observed gradations as to the quality of the different ranges. On some of the ranges it is impossible to maintain cattle; on others the condition of "salt sick" never occurs, while other areas are marginal.

The iron content of wire grass determined by the same authors, tends to decrease with advancing stages of growth. This tendency is more marked on burned over than on unburned ranges in Florida. Wire grass and other forage analyzed showed less iron when grown on "salt sick" areas than did that grown on areas not affected by "nutritional anemia".

Bryan and Becker (10), trying to correlate the mineral content of soil types to the "salt sick" condition of cattle, have found that soils in the healthy area contained over twice as much silt and clay (9.0%) in the first foot as did those in the "salt sick" area (4.3%), and over three times as much (15.2% as against 4.4%) in the second foot. The sick areas nearly always were of sandy or fine sand type with an average of 0.04% iron, while the healthy areas consisted of fine sandy loams with clay subsoils with an average of .422% iron.

In New Zealand, Aston (4, 5, 6, 7, 8) is the leader in the investigations carried out with "bush sickness" of livestock in that country. He demonstrated that this sickness was caused by a deficiency of iron. He showed that "bush sickness" or "nutritional anemia" was prevalent in both sheep and cows in parts of the counties of Rotorua, [illegible], and Tauranga in the North Island, New Zealand. Aston points out that the soils of the "sick" counties are invariably soils consisting largely of pumice and other "ejectamenta", scattered by the winds. This pumice has its origin in acidic lava and therefore contains a large percentage of silica. According to the same author, grasses and clover do well on the "bush sick" country, going through their life cycle perfectly, producing good sized plants with fertile seeds.

When Askew and Rigg (1) and Aston (5) analyzed samples of forages from affected and healthy areas in New Zealand, they found very little iron present in the forage from the affected area.

Aston (6) says that "if the pasture be top-dressed with an iron salt, the result is to decrease the clover and hence reduce the carrying-capacity. The net result, however, is that stock can be carried longer on this pasture than on any other, because they remain healthy on the iron-dressed pasture longer in spite of the decreased quantity of herbage". The same author (6) shows that when phosphates in the form of super phosphates or basic slag are used as top-dressing, the cattle can be grazed longer on a "bush sick" area than if no treatment were given. On the other hand, if lime (7) is added to the "sick" area, the disease is hastened and the cattle have to be treated or moved to healthy areas much sooner.

Aston reports (5) the total iron content of "sick soils" as varying from .33 to 1.13% with an average of .86%. In the healthy area, however, the total iron content varies from .81 to 2.04% with an average of 1.24% iron. The available iron extracted with 1% citric acid from the "sick soil" varies from .022 to .07% with an average of .043% available iron. In the healthy area the available iron varies from .078% to .318% with an average of 1.67%.

Grimmett and Shorland (15) also of New Zealand have shown that iron free hydrochloric acid extracts of Ruatangata limonite contained traces of cobalt and zinc. When the extracts were administered to sheep suffering from "bush sickness", they caused a slow improvement in the health of the animals. Similar results were obtained with pure solutions of cobalt acetate and zinc salts. On the other hand, the condition of "bush sick" sheep was definitely improved by the administration of ferric ammonium citrate which had been specially treated to remove cobalt and other traces of elements. According to the same author, it appears that traces of elements such as cobalt may exert a stimulating effect, or aid iron assimilation. They find, however, that iron is required and is curative.

[illegible] (11) another New Zealand worker, thinks that deficiency of cobalt in the soil is the real cause of "bush sickness" in New Zealand. His reports are based on a few initial studies and need much more investigation to be conclusive.

C. G. Dickinson (12) has shown that the so-called "coasty disease" of King Island, Tasmania, is the nutritional anemia and similar in origin to the "bush sickness" of New Zealand and the "salt sick" of Florida.

W. [illegible] (14) in 1928 and J. B. Orr (16) in 1931, both have shown in their publications that "pining" of sheep in the Cheviot Hills of Scotland and "nakuruitis" in the Nakuru district of Kenya Colony are also caused by a nutritional anemia or a deficiency of iron in the forage that the affected livestock eat.

THE PURPOSE OF THE INVESTIGATION.

The primary purpose of this research problem has been to see whether the iron content can be increased in grasses grown on soils from certain areas of the southeastern part of Massachusetts, where cattle have been reported to be affected with nutritional anemia. The secondary purpose of this investigation has been to study the tolerance of grass to the amounts and kinds of iron compounds added to a known silt loam, and the relationship existing between the reaction of that soil and the iron content of the hay grown upon it.

SOILS FROM AREAS PRODUCING DEFICIENT HAY.

In the fall of 1934 a quantity of representative soil was obtained from three farms in the area where nutritional anemia in cattle was prevalent. On two of these farms actual cases of the disease had been observed. In addition a corresponding amount of soil was taken as a check or control from a fourth farm in an area where no anemia in cattle had been reported. This soil in all respects may be considered as a normal or a healthy soil. The soils are listed below according to their given laboratory number and exact original location:

Laboratory Number	Soil obtained from	Town
2	Great Hill Farm	Marion
3	Herrick Estate	Wareham
4	C. H. Bryant	Wareham
5	L. C. Humphrey	Rochester

INVESTIGATIONS WITH SOUTHAMPTON SOILS.

At about the same time that work with the soils from Buzzards Bay region was started, a farmer in Southampton, Massachusetts, called upon certain members of the Massachusetts Agricultural Experiment Station to investigate a peculiar disease which was troubling his flock of sheep.

It seemed that his flock was not thriving very well and certain individuals within the flock were quite badly affected. During the initial investigation an iron salt drench was administered to the sick animals, and an apparent cure for the malady was obtained.

With the apparent success of these first trials it was decided to carry out experiments on the Southampton soils similar to those of the Buzzard's Bay region.

Samples of soil, laboratory numbers 10 and 20, were taken from the Southampton farm where the sick sheep had been reported. Soil number 10 was taken from a newly cultivated area while soil number 20 was taken from an old hay field.

Data of results obtained from the Southampton soils will be given throughout this paper in the same tables with data from the soils around Buzzards Bay region.

Sometime after the experimentation with these soils had commenced, further investigation by a certain member of the Experiment Station with the sick sheep, showed that perhaps the cause of this particular malady was due more to general nutritional deficiencies than to purely nutritional anemia.

The experimentations of the author with the Southampton soils were continued, however, and the results reported must be considered in the light that these soils, although low in iron, are not necessarily producing nutritional anemia, but more correctly, general nutritional deficiencies.

RESULTS OF INVESTIGATION WITH SOILS FROM AREAS SURROUNDING BUZZARDS BAY AND SOUTHAMPTON.

Eight pounds of each of the air dried soils were placed in individual one gallon crocks and given the treatments listed according to letters of the alphabet.

Treatment Designation	Treatment
A	nothing added
B	complete fertilizer
C	complete fertilizer plus ferric ammonium citrate
D	complete fertilizer plus iron oxide (Fe_2O_3)

The complete fertilizer added to the soil in the crocks that received the "B" treatment consisted of the following "C. P." chemicals:

1.52 grams of $NaNO_3$
1.18 grams of $(NH_4)_2SO_4$
.89 grams of $CaH_4(PO_4)_2 \cdot H_2O$
.93 grams of K_2SO_4

The soils in the crocks that got the "C" treatment received 1.46 grams of ferric ammonium citrate in addition to the complete fertilizer used in treatment "B". The "D" treatment consisted of .36 gram of Fe_2O_3 per crock in addition to the complete fertilizer.

A hay seed mixture of timothy and red top, in the proportion of four parts timothy to one part red top, was then sown evenly in each crock. The whole experiment was duplicated.

Below will be found Table I, which gives the per cent of coarse gravel (above 2 mm.), maximum water holding capacity and the pH of the soils studied.

TABLE I

Soil lab. No.	Coarse Gravel (per cent)	Max. H_2O holding Capacity	pH
2	1.1	48.	5.65
3	.8	37.	4.65
4	.4	51.	5.70
5	5.3	50.	5.60
10	5.2	51.	5.55
20	5.3	53.	6.00

Throughout the duration of the experiment the crocks were watered with distilled water sufficiently to establish a moisture content in the soils equal to about 50% of the maximum water holding capacity of each individual soil.

After allowing the grass to grow in the green house for seven months, until the stage of maturity was reached where no further upward growth was being made, the hay was harvested, with green weights and oven-dry weights recorded. A representative soil sample was taken at this time for available and total iron determination.

The dried grass was then finely ground in a Wiley mill. Any slight iron contamination from this source should be nearly uniform for all samples.

Analyses for iron were then performed on the ground grass samples by use of a micro colorimetric method. This method was tried and adopted in work done by Dr. R. O. Brooke, chemist in charge of the Wirthmore Research Laboratory, Malden, Massachusetts. Since this method for iron determination in plant tissue is a greatly modified one, the writer feels that a description of the procedure, as proposed by Dr. Brooke, is in order.

<u>Micro-colorimetric Method for Iron Determination in Plant Tissue.</u>

One-tenth of a gram (0.1 gram) of the finely ground grass is transferred into a 50 c.c. graduated Kjeldahl flask. 10 c.c. of sulphuric acid (1:1), 5 c.c. of 60% perchloric acid and a glass bead are added and the mixture brought to a boil over a micro-burner. Five drops of nitric acid are added from time to time until a clear solution is obtained. The digest is boiled for 30 minutes and allowed to cool.

The addition of five drops of Superoxol will oxidize any nitrous acid present. After boiling for a few minutes, the mixture is cooled to room temperature and finally diluted to the mark. A blank determination for iron in reagents is carried on simultaneously.

An aliquot of 10 c.c. is pipetted into a 50 c.c. glass stoppered cylinder, enough sulphuric acid added to bring the final acidity to about that of 1 N acid. The volume is then made up to 15 c.c. with distilled water, one drop of concentrated nitric acid, 0.5 c.c. of a 4% solution of potassium persulphate, and 10 c.c. of a mixture of one part of ethylene glycol monobutyl ether and one part of ethyl ether were added in the order given, and finally 5 c.c. of a 10 per cent potassium thiocyanate was run in. The contents of the cylinders were thoroughly shaken and allowed to stand five minutes.

By means of a 2 c.c. pipette a portion of the ethereal layer was quickly transferred to the cup of a Bausch and Lomb micro-colorimeter for comparison. The standard solutions containing a known amount of iron were set at 15 mm. and compared with the test solution.

A stock solution of iron was made by dissolving 0.817 grams of analytical iron wire in 100 c.c. of 10% sulphuric acid and diluted to 1000 c.c. after the addition of 3.0 c.c. of nitric acid. This solution was then diluted so that 2 c.c. would contain 0.01293 mgs. of iron.

Ten standards were then prepared, by running from a burette 4.0, 6.0 c.c. and so on up to 30.0 c.c. into different 50 c.c. flasks, adding 10 c.c. of sulphuric acid (1:1) to each and diluting to the mark with distilled water.

In making the comparisons in the colorimeter, it was found most convenient to manipulate four cylinders in a series. In each series, one cylinder contained a freshly prepared standard and the remaining three, test solutions. Six readings were made in each case. Therefore the three test solutions were individually compared with the same standard, which, however, was not allowed to remain in the cup, but freshly pipetted from the glass stoppered cylinder for each separate comparison.

METHOD FOR DETERMINING IRON IN THE SOIL.

The following procedure was adhered to in the determination of the available iron of the soil: To a five gram sample of soil, 50 c.c. of 5% oxalic acid was added and the mixture shaken for five minutes and allowed to stand over night (fifteen hours). It was then filtered through a number 40 Whatman filter paper, eleven cms. into a 250 c.c. volumetric flask. After washing ten times with 5% oxalic acid, the filtrate was made up to volume with distilled water. An aliquot of 25 c.c. was then taken, evaporated to dryness and the residue ignited at a low temperature in order to get rid of the excess of oxalic acid which would interfere with the development of the color in the final determination. The residue was taken up with HCl (1:6) and made up to a volume of 100 c.c. The known aliquot was then taken from this volume and iron determined as described above.

The total iron of the soil was determined by the accepted method of the Association of Official Agricultural Chemists (2).

DATA OF THE [illegible] WITH "SICK SOILS".

The yields and iron content of the grasses is reported in Table II along with the iron content of the soils.

In Table III is shown the per cent of increase of iron in the grass grown on iron treated soil (treatment C and D) over that grown on the untreated soil (treatment B). Table III also shows the increase of available iron in the iron treated soil over the untreated soil.

TABLE II

Soil and treatment number	Yields of Grass: Green wt. (grams)	Yields of Grass: Dry wt. (grams)	Fe in dry grass (%)	Gross amt. of Fe used up per treatment (grams)	Total Fe in soil (%)	Available Fe in soil (%)
2-A	15.95	8.51	.0257	.00219	.73	.02151
2-B	40.25	18.75	.0239	.00448		.08442
2-C	30.08	15.17	.0394	.00598		.10757
3-A	22.63	11.37	.0309	.00351	.52	.01637
3-B	60.09	26.48	.0404	.01070		.02348
3-C	58.73	28.20	.0492	.01387		.17646
4-A	13.43	7.08	.0232	.00164	.37	.03650
4-B	60.63	29.03	.0254	.00737		.04368
4-C	54.52	28.41	.0369	.01048		.15544
5-A	22.99	11.46	.0294	.00337	1.88	.05983
5-B	58.89	29.62	.0248	.00735		.07834
5-C	58.38	29.16	.0468	.01365		.20390
10-A	5.13	2.24	.0255	.000571	1.99	.08623
10-B	31.91	14.80	.0221	.00327		.09939
10-C	48.51	18.00	.0150	.00270		.21058
10-D	37.67	15.03	.0190	.00286		.19791
20-A	7.88	4.28	.0218	.000933	2.16	.07463
20-B	40.12	17.44	.0181	.003157		.10840
20-C	35.18	16.91	.0201	.00340		.16945
20-D	32.20	16.15	.0121	.00195		.14349

Legend of Treatments: A = nothing added; B = complete fertilizer; C = complete fertilizer plus ferric ammonium citrate; D = complete fertilizer plus iron oxide.

TABLE III

PERCENTAGE INCREASE OF IRON FOUND IN GRASSES AND IN SOILS.

Soil and treatment number	Increase of Fe in grass in treatments C and D over treatment B (%)	Increase of available Fe in soil in treatments C and D over treatment B (%)
2-C	64.9	27.4
3-C	21.8	651.5
4-C	45.3	255.9
5-C	88.7	160.3
10-C	-32.1	111.9
10-D	-14.0	90.1
20-C	11.1	86.3
20-D	-32.2	32.4

DISCUSSION OF RESULTS.

In Table II it will be noted that the check crocks (treatments "A"), which did not receive any applications of fertilizer or iron, produced a very low yield of dried hay. The increase of dry weight of the hay of treatments "B", varies approximately from two to seven times that found in the "A" treatments. The large increase in yield due to the fertilizer applications points conclusively to the very low fertility of all the soils used, whether they were obtained from "sick" areas or "healthy" areas.

Although in soils number 3 and number 10 there was an increase of dry weight of hay with the iron application, the slight decrease of weight in the other four soils is not significant enough to conclude that the iron application, at the rates used, had any ability to either increase or decrease the dry weight of hay.

With the exception of soils number 3 and number 4, which are extremely low in total iron, it will be noted that the per cent of iron found in the plant tissue is greater in the grasses grown on the untreated soils. This may be partly caused by the very slow and small, abnormal growth of the grasses in the untreated crocks.

Grasses grown on soils from the Buzzard's Bay regions show a definite large increase of iron due to the iron application when comparisons are made of cases where other plant foods are not the limiting factors. In the case of the Southampton soils, which are not exceptionally low in total and available iron content, there is a general decrease of the per cent of iron found in the grasses grown on the iron treated series.

Table III shows the increases or decreases of iron content with the grasses having an iron application. Of the Buzzards Bay soils having the iron application, the greatest increase of iron (83.7%) was found in soil number 5, which is not an anemic producing soil. Soil number 3 with only an increase of 21.8% had the lowest per cent increase. In the Southampton soil, where two forms of iron were used in the iron treatment, no definite correlation could be established as to which form of iron was better. In soil number 20, the inorganic form was taken in the least, while with soil number 10 the organic form showed the least absorption.

The column in Table II which shows the gross amount of iron absorbed by the growing grasses is very interesting. This set of figures was obtained by multiplying the dry weight of hay grown on each treatment by the corresponding percentage of iron that was found in the hay grown upon it. It will be noted that the gross amount of iron used up by the grass grown on the "C" treatments, exceeds greatly the "B" treatments, and exceeds even more the "A" treatments, in the case of the Buzzards Bay soil. Again there is no correlation with the Southampton soils. It may be concluded that these Southampton soils, which are naturally high in available and total iron, show no increase of absorption or iron by the plant when iron was applied.

The per cent of total iron found in soils numbers 2, 3, 4, 5, 10 and 20 before any treatment was applied to them was .73, .52, .37, 1.88, 1.99 and 2.16 respectively. The "healthy" soils numbers 5, 10 and 20 contained several times more iron than the soils numbers 2, 3 and 4 from the "sick" area. Although the total iron content of the Massachusetts soils from the "sick" area was considerably higher than that of Florida's "sick soils" as reported by Becker (10), the analyses of the New Zealand soils are quite similar (4).

Table II also shows the per cent of available iron found in the different soils at the time that the grass crop had been harvested. It was found that the available iron (5% oxalic acid extract) of the "sick" soils was much lower than that of soils numbers 5, 10 and 20. For some reason the application of fertilizer alone ("B" treatment) had increased the available iron over that found in the unfertilized series ("A" treatment). This increase was probably due to three causes. (1) The added fertilizer chemicals, although of "C. P." purity, contained a trace of iron. (2) The fertilizer, especially the potassium, may have released and made more available some of the iron. (3) The increase of plant growth in the fertilized series over the unfertilized had without any doubt greatly increased the organic matter of the soil because of added root growth. This increased organic matter probably contained iron that was extracted in making the available iron determination.

The per cent increase of available iron of treatments "C" and "D" over treatments "B" has been reported in Table III. The increase of available iron has generally been greatest with the "sick" soils from the Buzzard's Bay region. These soils were much more sandy in texture and thus contain a smaller amount of colloidal fraction which probably tends to tie up the added iron.

In generalizing the data of the results reported in Tables II and III, one can say that the iron content of the grasses grown on soils from the "sick" areas surrounding Buzzards Bay region, can be increased with application of soluble iron salts to the soil. This above generalization does not hold for the grasses grown on the Southampton soils, for with them only in one instance was there a slight increase.

METHOD OF INVESTIGATION WITH A GIVEN SILT LOAM.

As stated in the beginning of this paper, the second part of this research deals with the possibility of increasing the iron content of grasses when they are grown on a given soil treated with different iron compounds at different rates of application. Although much literature has been published on the role of iron and its correlations to toxicity in plants, pH of media of growth, kinds of iron compounds used, rate of its applications and crop yields; no work has yet been done to determine if the iron content of grasses can be increased by the addition of increased increments of iron to the soil.

A brown silt loam of the Merrimac Series, analyzing 3.51% total iron, was used throughout this investigation. In one series of experiments the soil pH was 4.4 and in the other the pH was changed to 5.4 by liming the area from which the soil was taken, for several years previously.

Eight and one-half pounds of this soil were placed in one gallon crocks to which was added either ferric oxide (Fe_2O_3), ferric sulphate ($FeSO_4 \cdot 7H_2O$), or ferrous sulphate ($Fe_2(SO_4)_3 \cdot xH_2O$) at rates of 10, 20, 40, 80, 160, 320, 640, 1280, 2560, and 5120 pounds per acre. A complete fertilizer was applied uniformly to all the crocks. Spinach, turnips and mixed hay grasses were grown in succession on the same soil. The moisture of the soil was kept at approximately 50% of the maximum water holding capacity of the soil with tap water. It was considered that the small accumulation of iron due to the tap water was insignificant when such large amounts of iron compounds were applied previously to the soil as treatments. Yields (on oven dry basis) were taken of each crop and notation made of the toxic concentrations for the different iron compounds used.

TOXICITY RANGE OF THE DIFFERENT IRON COMPOUNDS.

Table IV shows the concentrations at which iron toxicity definitely appeared in the three different crops.

TABLE IV

Crop	Soil pH	pound per acre causing injury		
		Ferric oxide	Ferric sulphate	Ferrous sulphate
Spinach	4.4	...*	640	1,280
	5.4	...*	1,280	5,120
Turnips	4.4	1,280	320	640
	5.4	...*	1,280	2,560
Mixed hay grasses	4.4	2,560	1,280	2,560
	5.4	...*	1,280	5,120

* Not toxic in concentrations used.

YIELDS OF CROPS GROWN

The oven dry weights of the different crops grown on the soils having a pH 4.4 and pH 5.4 are shown in Tables V and VI respectively.

TABLE V

YIELDS OF CROPS GROWN ON THE SOIL HAVING pH 4.4.

Weights reported on oven dry basis.

Treatment	Rate of application of treatment. (lbs. per acre)	Kind of Crop Grown: Spinach (grams)	Turnips (grams)	Mixed Hay (grams)
Fe_2O_3	10	.194	.593	3.671
	20	.196	.680	3.201
	40	.214	.737	3.483
	80	.235	.555	3.625
	160	.317	.556	3.746
	320	.285	.501	4.981
	640	.267	.493	4.350
	1280	.267	.416	4.252
	2560	.251	.420	4.006
	5120	.202	.4[illegible]1	3.583
$FeSO_4 \cdot 7H_2O$	10	.208	.588	3.301
	20	.262	.335	3.598
	40	.271	.629	3.896
	80	.283	.640	4.187
	160	.255	.779	5.823
	320	.234	.536	5.729
	640	.226	.432	4.312
	1280	.205	.135	3.721
	2560	.118	.000	2.978
	5120	.016	.000	.000
$Fe_2(SO_4)_3 \cdot xH_2O$	10	.274	.299	4.046
	20	.239	.493	4.356
	40	.282	.594	5.360
	80	.282	.794	5.048
	160	.286	.866	4.323
	320	.335	.525	4.794
			.126	3.948
	640	.209	.000	1.936
	1280	.198	.000	.000
	2560	.030	.000	.000
	5120	.009		
Check (no iron added)		.202	.613	3.958

TABLE VI

YIELDS OF CROPS GROWN ON SOIL HAVING pH 5.4.

Weights reported on oven dry basis.

Treatment	Rate of application of treatment. (lbs. per acre)	Kind of crop grown: Spinach (grams)	Turnips (grams)	Mixed Hay (grams)
Fe_2O_3	10	1.497	1.477	7.021
	20	1.485	1.215	7.201
	40	1.327	1.196	6.655
	80	1.306	1.200	6.551
	160	1.309	1.192	6.505
	320	1.275	1.098	6.424
	640	1.307	.921	6.545
	1280	1.257	.911	5.923
	2560	1.251	.800	5.686
	5120	1.203	.776	5.224
$FeSO_4 \cdot 7H_2O$	10	1.104	.847	6.112
	20	1.083	.930	6.259
	40	1.207	1.016	6.253
	80	1.199	1.336	6.718
	160	1.552	1.290	6.879
	320	1.607	1.659	6.228
	640	1.499	1.052	5.676
	1280	1.502	1.168	5.595
	2560	1.305	.850	5.253
	5120	.616	.818	4.912
$Fe_2(SO_4)_3 \cdot xH_2O$	10	1.776	1.157	6.594
	20	1.794	.905	6.935
	40	1.657	.979	6.369
	80	1.657	.864	6.124
	160	1.595	.832	5.935
	320	1.530	.886	6.007
	640	1.547	.795	5.777
	1280	1.369	.800	5.278
	2560	1.284	.684	4.873
	5120	.161	.338	4.5[illegible]0
Check (no iron added)		1.287	.794	6.330

AMOUNTS OF IRON FOUND IN THE HAY.

Since the writer was primarily interested in the possibility of increasing the iron content of grasses, no analyses for iron were made on the spinach or turnip. In order to save time in the analytical work on the dried grasses, only those grasses were analyzed which were grown on every other rate of iron application. Thus, in Tables VII and VIII are reported only the iron analyses of hay grown on soil receiving the following rates of application of the different iron compounds: 20, 80, 320, 1280, and 5120 pounds per acre.

TABLE VII

IRON CONTENT OF HAY GROWN ON THE SOIL HAVING pH 4.4.

Percentages reported on oven dry basis.

Treatment	Rate of application of treatment (lbs. per acre)	Dry wt. of hay (grams)	Fe found (%)	Gross amt. Fe used up by hay (mg.)	Increase of Fe over check (%)
Fe_2O_3	20	3.201	.0256	.819	12.7
	80	3.625	.0310	1.123	36.7
	320	4.981	.0330	1.644	45.4
	1280	4.252	.0326	1.386	43.6
	5120	3.583	.0250	.896	10.1
$FeSO_4 \cdot 7H_2O$	20	3.592	.0234	.841	3.0
	80	4.197	.0240	1.049	5.7
	320	5.729	.0260	1.490	14.5
	1280	3.721	.0221	.822	- 2.6
	5120	.000	-----	---	---
$Fe_2(SO_4)_3 \cdot xH_2O$	20	4.356	.0269	1.172	18.5
	80	5.048	.0364	1.837	60.4
	320	4.704	.0362	1.702	59.5
	1280	1.936	.0366	.709	61.2
	5120	.000	-----	---	----
Check (no iron added)		3.958	.0227	.898	

TABLE VIII

IRON CONTENT OF HAY GROWN ON THE SOIL HAVING pH 5.4.

Percentages reported on oven dry basis.

Treatment	Rate of application of treatment (lbs/acre)	Dry wt. of hay (grams)	Fe found (%)	Gross amt. of Fe used up by hay (mg.)	Increase of Fe over check (%)
Fe_2O_3	20	7.301	.0183	1.336	10.2
	80	6.531	.0295	1.923	77.7
	320	6.424	.0303	1.946	82.5
	1280	5.923	.0547	3.240	229.5
	5120	5.224	.0467	4.007	181.3
$FeSO_4 \cdot 7H_2O$	20	6.259	.0200	1.252	20.5
	80	6.718	.0268	1.800	61.4
	320	6.228	.0286	1.781	72.3
	1280	5.595	.0159	.890	- 4.2
	5120	4.912	.0114	.560	-31.3
$Fe_2(SO_4)_3 \cdot xH_2O$	20	6.935	.0260	1.803	56.6
	80	6.124	.0352	2.156	112.0
	320	6.007	.0402	2.415	142.1
	1280	5.278	.0602	3.177	262.7
	5120	4.520	.0500	2.260	201.2
Check (no iron added)		6.330	.0166	1.051	

DISCUSSION OF RESULTS.

An outstanding difference was noted in the concentrations of different iron compounds causing toxic injury to plants in soils of different reactions. The appearance of toxic conditions to the plants was observed at lower rates of applications on the more acid soil. The ferric oxide treatments were the least toxic and decreased the plant growth only in the more acid soil, when the higher rates of application were used with turnips and mixed hay grasses.

The ferric sulphate showed signs of toxicity at the lower concentrations of iron than either the ferric oxide or ferrous sulphate. The reason for the toxic concentration being lower with ferric sulphate was due probably not to the iron content of the salt as much as to the greater percentage of sulphate ion that the salt contains. From Table IV, it is found that mixed hay grasses withstood higher concentrations of added iron compounds, while the turnips showed signs of toxicity at much lower concentrations.

Tables V and VI show that the soil with the higher pH produced larger dry weights of the different crops. This increase was due to the higher pH being a better suited one for the different crops used. The dry weights of spinach grown on the soil with the higher pH were generally about seven times greater than those grown on the soil with the lower pH. In the case of the turnips and hay, on the other hand, dry weights were about twice as large.

In the case of the more acid soil the yield with all iron treatments increased gradually as the concentrations increased until an optimum concentration was reached. Any increased concentration of iron over this optimum concentration tended to decrease the yield of the crops until a toxic concentration was reached. The optimum concentration range of the three crops for ferric oxide, ferric sulphate and ferrous sulphate was between 40 to 320 pounds, 60 to 160 pounds, and 40 to 320 pounds respectively.

A somewhat similar situation, was found in the case of the soil having a pH of 5.4. In the series where ferric oxide was used, the three crops were apparently stimulated and produced highest yields with the lower concentrations of added iron. Although no toxic concentrations were experienced, the yields showed a definite decrease with increasing concentrations of ferric oxide.

The concentrations of ferric sulphate that showed the optimum growth range for the three crops was about 80 to 160 pounds per acre. Increased concentrations beyond this range decreased the yield until a toxic concentration was reached. The ferric sulphate acted similarly to the ferric oxide except that a definite toxic concentration was reached at the 1280 pounds concentration with each crop.

In both soils the yields of the three crops at the optimum concentrations were much larger than the checks which did not have any iron added to them. The yields of the crops grown on the less acid soil that received the lowest rate of application were higher than the corresponding checks. These relations did not exist with the other soil, for the yield of the check in many cases was approximately equal or slightly less.

Table IX shows these concentrations of iron added to the soils of both reactions which were considered optimum, as judged by the largest yields.

TABLE IX

Crop	Soil pH	Pound per acre causing optimum growth		
		Ferric oxide	Ferric sulphate	Ferrous sulphate
Spinach	4.4	160	320	80
	5.4	10	10	320
Turnips	4.4	40	160	160
	5.4	10	10	320
Mixed Hay Grasses	4.4	320	40	160
	5.4	20	20	160

No outstanding relationship was found in the iron content of hay grown on the two soils with the different soil reactions. This was probably due to the large differences between two successive rates of application of the different iron compounds. From the study of Table VII, it was concluded, in a general manner, that the iron content of hay grown on the more acid soil tended to increase in the greatest amounts at the concentrations of added iron where the largest dry weight of hay was grown. With the exception of ferric sulphate series, where the difference was considered insignificant, the above relationship appeared to be the same for the different iron treatments. In the case of the ferric oxide and ferrous sulphate treatments any further increase of the rate of application tended to decrease the per cent of iron toward that found in the check.

The results in Table VIII show that the greatest per cent of iron in the hay for the ferric oxide, ferrous sulphate and ferric sulphate treatments, was found in the 1,280, 320 and 1,280 concentrations respectively. It must not be over looked that there may have been a point on either side of the above concentrations where a higher iron content was possible, but, since the hay grown on the intermediate concentrations was not analyzed, their yields can not be used in drawing conclusions. A decrease of iron compared with that of the check was found with both soils when the ferrous sulphate was used in the higher concentrations that were toxic. The ferric sulphate treatment seemed to result, generally, in a larger gross absorption of iron than either of the other iron compounds used.

With the exception of the concentrations where the ferrous sulphate caused a toxicity, as mentioned above, the iron content was greatly increased over the checks on both soils. The greatest increases were with the limed soil.

<u>SUMMARY</u>.

The cause, symptoms, cure and distribution of nutritional anemia in livestock has been presented by a study of the literature. This particular disease of livestock is of great economic importance in many different parts of the world.

Certain soils that produced a nutritional anemia in Massachusetts cattle had an iron compound added to them. Mixed hay grasses were grown and analyzed for their iron content. Iron applications, at the rates used, had no significant ability of either increasing or decreasing the dry weight of hay. All of the "sick" soils from the Buzzards Bay region showed a definite large increase of iron due to the iron application when comparisons were made of cases where other plant foods were not the limiting factors.

Iron salts added to a series of Southampton soils, with one exception, decreased the iron absorbed by the grasses grown upon them.

With one exception, found in the Southampton soil, the gross amounts of iron used up by the grasses has been increased when grown on the different soils.

It has been found that the per cent of available iron (5% oxalic acid extract) in the soil can be increased slightly by fertilization. Large increases of available iron were found when ferric ammonium citrate was applied to the soil.

The per cent of total iron found in the Buzzards Bay soils numbers 2, 3, 4 and 5, and in the Southampton soils numbers 10 and 20, was .73, .59, .57, 1.38, 1.99, 2.16 respectively.

In order to find the toxic concentrations of different iron compounds, they were applied in increasing rates to a given silt loam, and various crops were grown in succession. Dry weights and appearance of the crop was used as a criterion in establishing the toxic concentrations. It was found that the ferric sulphate showed signs of toxicity at lower concentrations of iron than either ferric oxide or ferrous sulphate.

The soils with a pH 5.4 produced much greater dry weights of the different crops than the same soils with a pH of 4.4.

A definite concentration for each of the different iron compounds used was found which produced an optimum growth of the various crops grown.

With the exception of those concentrations of ferrous sulphate which caused toxicity, as shown in Table VIII, the iron content of the hay was greatly increased over that of the checks when iron compounds were applied at different rates to a given soil. The largest increases of iron were found with the soil having a higher pH value.

LITERATURE CITED

1. Askew, H. O., Rigg, T., Grange, L. I., and Taylor, N. H.
Bush Sickness, Investigations Concerning the Occurrence and Cause of Bush Sickness in New Zealand.
New Zeal. Dept. Sci. and Indus. Res. Bul. 32, 1932.

2. Association of Official Agricultural Chemists.
Official Methods of Analyses.
Third Edition, 1930.

3. Aston, B. C.
Bush-Sickness Investigation. Five Years' Work at the Mamaku Demonstration Farm.
New Zeal. Jour. Agr. 28: 215-238, 1924.

4. ———— The Bush-Sickness Investigation.
Laboratory Work and Results.
New Zeal. Jour. Agr. 28: 301-305, 381-390; 29: 84-91, 1924.

5. ———— Mineral Content of Pastures. Progress of the New Zealand Investigation.
New Zeal. Jour. Agr. 36: 22-27, 75-80, 1928.

6. ———— Cure of Iron Starvation in Stock.
New Zeal. Jour. Agr. 40: 223-227, 1930.

7. ———— The Liming of Land with Special Reference to the Bush-sick Lands.
New Zeal. Jour. Agr. 49: 51-56, 80-85, 1934.

8. Aston, B. C., Grimmett, R. E. R., Brogan, F. J. A. and Byrne, P. M.
Mineral Content of Pastures. The Wairarapa District.
New Zeal. Jour. Sci. and Technol. 13: 304-320, 1931.

9. Becker, R. B., Neal, W. M. and Shealy, A. L.
I. Salt Sick: Its Cause and Prevention.
II. Mineral Supplements for Cattle.
Fla. Agr. Exp. Sta. Bul. 231, 1931.

10. Bryan, O. C. and Becker, R. B.
The Mineral Content of Soil Types as Related to "Salt Sick" of Cattle.
Jour. Am. Soc. of Agron. vol. 27, No. 2, 120-127, 1935.

11. Dixon, J. K.
Cobalt Investigation in New Zealand.
Science, vol. 85, No. 2207: 301, 1937.

12. Dickinson, C. G.
King Island "Coasty" Disease.
Aust. Vet. Jour. 3: 83-85, 1927.

13. Grimmett, R. E. R. and Shorland, F. B.
Use of Limonite in Bush-Sickness.
New Zeal. Jour. Agr. 50: 367, 1935.

14. Gunn, R.
Pasture Investigation in Kenya. Notes on "Nakuruitis".
Kenya Colony Dept. Bul. 545, 1928.

15. Neal, W. M., and Becker, R. B.
The Composition of Feedstuffs in Relation to Nutritional Anemia in Cattle.
Jour. Agr. Res. 47: 249-255, 1933.

16. Orr, J. B. and Holm, A.
The Mineral Deficiencies of Pastures in Kenya Colony and Their Effects on Grazing Animals.
(Gr. Brit.) Economic Advisory Council Com. on Mineral Content of Natural Pastures Rept. 6: 22-25, 45-48, 50-53, 1931.

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